

EXPERIMENTAL REGIME #1

Experimental regimes at the STA-1E Periphyton Test Facility are primarily defined by establishment of water depth, and residence time/hydraulic loading rate.

During Experimental Regime #1 (3-20-03 through 4-25-03) the following parameters were maintained in Cells 1, 2, and 4: Cell 3 was removed from the experiments because the Calcareous Periphyton mats could not be effectively established. Cattails dominated test cell 3 until it was flooded. Cell 3 will be placed into another substrate.

- **Water Depth – 6 inches (.5 ft.)**

PHOSPHOROUS RESULTS

This section contains phosphorous concentration data collected during the period from March 20, 2003 until April 25, 2003. The objective during Experimental Regime #1 was to compare the impact of the three different substrates/substrate mixtures on phosphorous uptake and periphyton community composition. The three substrates compared were 1) Cell 1 contains a 12-inch Riviera Sand with a thin coating of water treatment sludge at a spreading rate of 4 in³/ft² (100 ft³/acre). 2.) Cell 2 contains a 12 inch blanket of crushed limestone, 3) Cell 4 contains a 6-inch blanket of crushed limestone overlaying a 6-inch blanket of peat soils. All substrates were subjected to the same nutrient levels and hydrologic regime.

Total phosphorous samples were collected using two distinct methods within the test cells: 1) compositing of 12 water samples per week collected by a time-paced autosampler and 2) transect grab samples collected down the center of each cell every 15 feet. Samples were also collected before entering the test cells using autosamplers and were composited on a weekly or biweekly basis depending on logistics. Two sampling points were available 1) water entering the site from the C-51 canal and 2) water flowing into the test cells after allowing for settling in pools containing water hyacinth. Due to the high phosphorous concentration with C-51 all feed to the test cells were first pretreated within the water hyacinth pre-treatment pools. C-51 phosphorous concentrations averaged at about 100 ppb with spikes as high as 1000 ppb TP.

INPUT

| Week # | Sample Compositing Period | Location | Total Phosphorous (µg/l) |
|--------|---------------------------|-----------------|--------------------------|
| 1/2 | 3/20/03 - 4/03/03 | from C-51 canal | 88 |
| 3 | 4/04/03 - 4/11/03 | from C-51 canal | 81 |
| 4/5 | 4/12/03 - 4/25/03 | from C-51 canal | 74 |
| 1 | 3/20/03 - 3/27/03 | to test cells | 66 |
| 2 | 3/28/03 - 4/03/03 | to test cells | 71 |
| 3 | 4/04/03 - 4/11/03 | to test cells | 55 |
| 4/5 | 4/12/03 - 4/25/03 | to test cells | 69 |

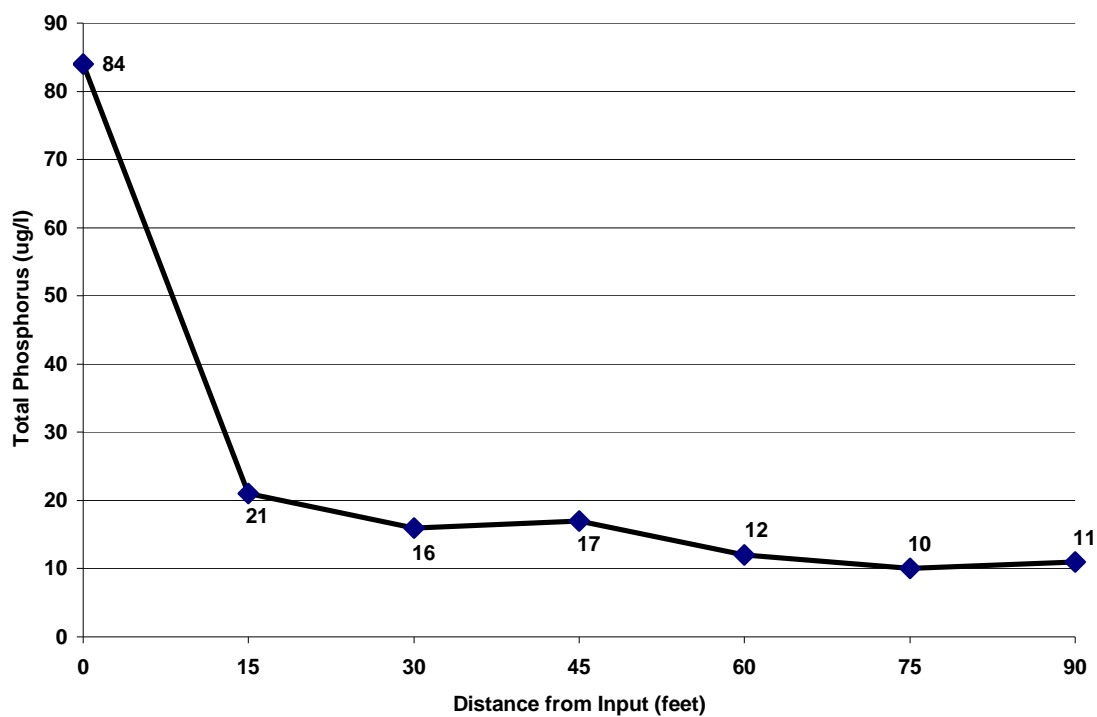
CELL ONE

Substrate: 12 inches of Riviera Sand w/ Water Treatment Sludge (4 in³/ft²)
Water Depth: 6 inches
HRT: 14 days
HLR: 1.06 cm/day

Total Phosphorous Concentrations Composited from ISCO Autosamplers

| Week # | Sample Compositing Period | Total Phosphorous (µg/l) |
|----------|---------------------------|--------------------------|
| 1 | 3/20/03 - 3/27/03 | 19 |
| 2 | 3/28/03 - 4/03/03 | 14 |
| 3 | 4/04/03 - 4/11/03 | 16 |
| 4 | 4/12/03 - 4/18/03 | 13 |
| 5 | 4/19/03 - 4/25/03 | 12 |

Transect Phosphorous Concentrations in Cell One on 4-25-03. Water was collected via grab samples every 15 feet along the centerline of the cell.



CELL TWO

Substrate: 12 inches of Crushed Limestone

Water Depth: 6 inches

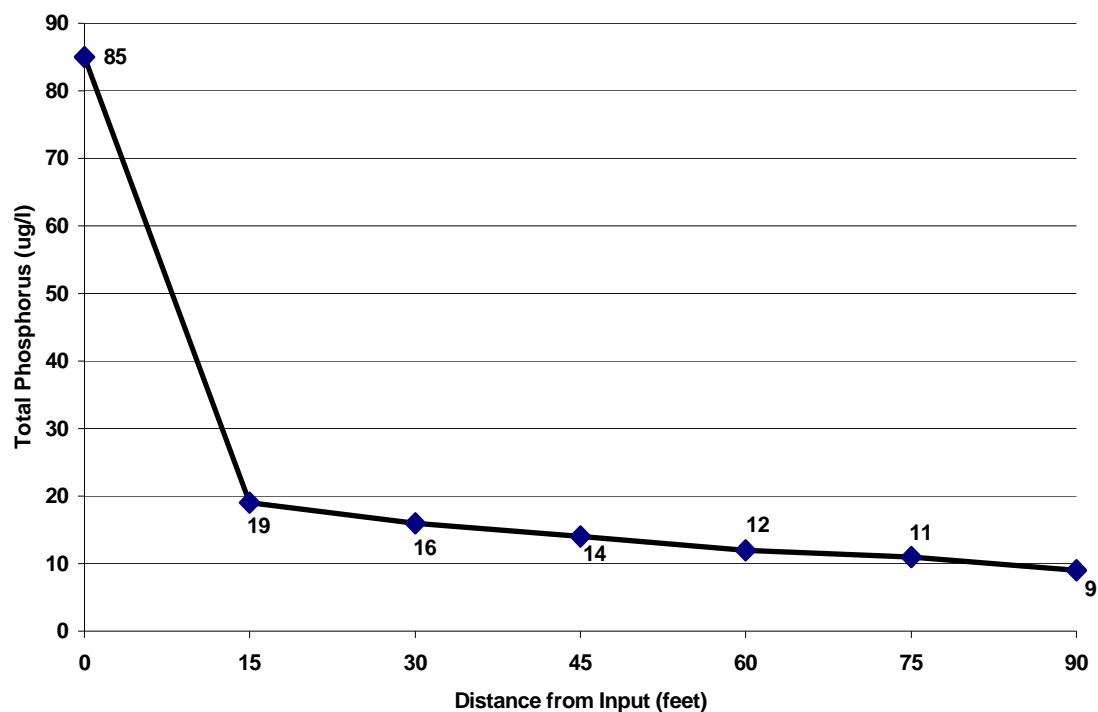
HRT: 14 days

HLR: 1.06 cm/day

Total Phosphorous Concentrations Composited from ISCO Autosamplers

| Week # | Sample Compositing Period | Total Phosphorous (µg/l) |
|--------|---------------------------|--------------------------|
| 1 | 3/20/03 - 3/27/03 | 10 |
| 2 | 3/28/03 - 4/03/03 | 10 |
| 3 | 4/04/03 - 4/11/03 | 10 |
| 4 | 4/12/03 - 4/18/03 | 10 |
| 5 | 4/19/03 - 4/25/03 | 10 |

Transect Phosphorous Concentrations in Cell Two sampled on 4-25-03. Water was collected via grab samples every 15 feet along the centerline of the cell.



CELL FOUR

Substrate: 6 inches of Crushed Limestone above 6 inches of peat soils (High Organic Matter)

Water Depth: 6 inches

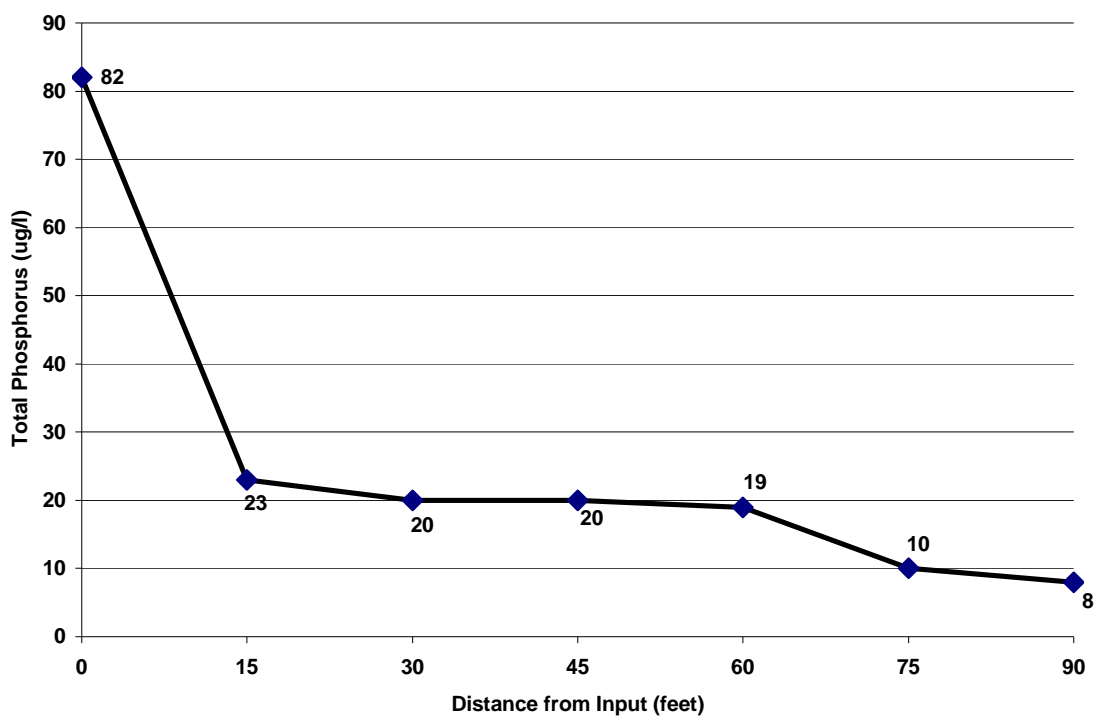
HRT: 14 days

HLR: 1.06 cm/day

Weekly Phosphorous Concentrations Composited from ISCO Autosamplers

| Week # | Sample Compositing Period | Total Phosphorous (µg/l) |
|--------|---------------------------|----------------------------|
| 1 | 3/20/03 - 3/27/03 | 10 |
| 2 | 3/28/03 - 4/03/03 | Control System Malfunction |
| 3 | 4/04/03 - 4/11/03 | 9 |
| 4 | 4/12/03 - 4/18/03 | 10 |
| 5 | 4/19/03 - 4/25/03 | 9 |

Transect Phosphorous Concentrations in Cell Four sampled on 4-25-03. Water was collected via grab samples every 15 feet along the centerline of the cell.



CELL PHOTOGRAPHS

Four per page – I will include later as this would be too large to email....will be organized by date and location

ACTIVITY LOG

3-20-03

- ISCO autosamplers restarted
- Cell 4 valves not working. Flow to cell 4 stopped to prevent excessive water depth.

3-25-03

- Water levels were at 1.5 feet on arrival at the site due to confusion between Cell 3 and Cell 4. Water levels returned to .5 feet. Samples for this week were not taken in this cell.
- Auto-recharge system installed for ISCO autosamplers
- Input flowmeters rinsed and cleaned

4-3-03

- Composite samples collected from ISCO autosamplers.

4-7-03

- Digital photographs of active cells taken.

4-9-03

- Cell water depths offsets reset.

4-11-03

- Composite samples collected from ISCO autosamplers
- Input flowmeters rinsed and cleaned

4-23-03

- Fine adjustments (.01 feet) made to water depth parameters so that all cells maintain the same depth.

- Input flowmeters rinsed and cleaned

4-25-03

- Composite samples collected from ISCO autosamplers
- Digital photographs of active cells taken.

DATA VALIDATION

The PSTA phosphorous samples were analyzed by Florida International University (FIU). The FIU laboratory has been certified in the State of Florida and has performed satisfactorily in the Round Robins conducted over the years. Listed below is the phosphorous analysis procedure established by Dr. Ronald Jones and used at FIU.

1- OBJECTIVE

This test procedure applies to the determination of total phosphorus in water, soil, sediment, and tissue samples.

2- PROCEDURE

For the determination of total phosphorus in water, soil, sediment, and tissue samples, SERP does not use the typical ammonium persulfate digestion because of the explosive hazards and special handling requirements associated with the use of this chemical. Instead, SERP uses a modification of the sample preparation methods described by Solórzano and Sharp (1980, *Determination of total dissolved phosphorus and particulate phosphorus in natural waters*. Limnol. Oceanogr., 25(4), pp. 754-758) (attached). Total phosphorus is determined in water, soil, sediment, and tissue samples by oxidizing and hydrolyzing all of the phosphorus-containing compounds in a sample to soluble reactive phosphate. Soluble reactive phosphate then is determined by reacting phosphate with molybdenum (VI) and antimony (III) in an acid medium to form a phosphoantimonymolybdenum complex; this complex is reduced with ascorbic acid to form a colored dye.

3- APPARATUS

Analysis for soluble reactive phosphorus is performed by wet chemical analysis using a two-channel Alpkem Rapid Flow Analyzer (RFA; Alpkem Corp., Clackamas, OR) Model 510 with Sampler Model 301 (See Figure 1) following EPA Method 365.1 and the procedure suggested by the Alpkem Corporation, modified for optimum conditions in our laboratory. SERP analyzes total phosphorus on a separate autoanalyzer from that used for soluble inorganic nutrient analysis.

4- SAMPLE PREPARATION

4.1- Sample Preparation Reagents

Magnesium sulfate (MgSO_4), 0.17 N: 10.475 g magnesium sulfate is dissolved in 250 ml of deionized water (DIW). 0.5 ml concentrated sulfuric acid is then added.

Hydrochloric acid (HCl):

0.06 N - 5 ml conc. hydrochloric acid in 1 L total volume DIW.

0.12 N - 10 ml conc. hydrochloric acid in 1 L total volume DIW.

0.18 N - 15 ml conc. hydrochloric acid in 1 L total volume DIW.

0.24 N - 20 ml conc. hydrochloric acid in 1 L total volume DIW.

Fig.1

(Use caution when making acid solutions. Always add acid to water. The mixing of acid in water may generate heat.)

4.2- Matrix Spike Sample Preparation

50 ml from the samples selected in the field (1 per sample set or 5%, whichever is greater) to be used as matrix spike samples (See SOP 003-98) will be transferred to another bottle and spiked with 200 μl of the SOP 002-98 Mixed Standard (0.31 μmol of P /ml) yielding a final spiked P concentration of 1.25 μM . These matrix spike samples will be run by duplicate.

4.3- Water Sample Preparation

Water sample preparation for total phosphorus determination should be done within 24 hours of sample collection:

1. Prepare a tray with two 8 ml scintillation vials without the aluminum-lined caps per sample bottle (four vials per sampling site) plus two vials of method blanks per every 10 sample set , and two vials for each matrix spike. Add 100 μl of 0.17 N MgSO_4 to each vial using the 5.0 ml dispenser tip (1 = 100 μl) and an Eppendorf pipet setting of 1. Fill out a total phosphorus preparation log sheet (see attached) with the placement and contents of the vials on the tray. Record the date the MgSO_4 used was made.
2. Add 5 ml of sample water into each of the vials (except for the method blank vials).
3. Place tray in an 80 $^{\circ}\text{C}$ oven and evaporate to dryness (usually 48 h). Record the date and time the samples were placed in the oven and removed from the oven on the log sheet.
4. Transfer the vials from the plastic trays to the metal trays, keeping the vials in the exact same order as outlined on the sample preparation log sheet. Ash the samples at 550 $^{\circ}\text{C}$ in a muffle furnace for 3 hours and allow to cool overnight. A melt pellet (melting point of 550 $^{\circ}\text{C}$) should be placed in an empty vial in an empty space on one of the trays to confirm that the furnace reached 550 $^{\circ}\text{C}$. Record the date and time the samples were placed in the

furnace and removed from the furnace and whether or not the pellet melted. Once cool, return the vials from the metal trays to the plastic trays, keeping the vials in the exact same order as outlined on the sample preparation log sheet.

5. Hydrolyze each sample with the addition of 5 ml of hydrochloric acid. The normality of the acid is dependent on the salinity of the sample according to the following table:

| <u>Salinity</u> | <u>HCl concentration</u> |
|-----------------|--------------------------|
| 0.0 - 15 ppt | 0.06 N |
| 16 - 38 ppt | 0.12 N |
| 39 - 55 ppt | 0.18 N |
| 56 - 80 ppt | 0.24 N |

Record the date and time the acid was added, the acid concentration(s) used, and the date(s) the acid(s) was/were made on the sample preparation log sheet. The method blanks are hydrolyzed with the 0.06 N HCl.

6. Cap each sample tightly with polylined caps, shake using a vortexer, and put into an 80 °C oven overnight. After removing from the oven, allow to cool and shake again. Record the dates and times the vials were taken out of the oven and shaken on the sample preparation log sheet.

4.4- Soil, Sediment and Tissue Sample Preparation

1. Dry sample in an 80 °C oven for 2 days, then grind.
2. Prepare a tray with two 7 ml glass scintillation vials (without the aluminum-lined caps) per sample plus two vials of method blanks, and two vials for citrus leaf standards. Fill out a total phosphorus preparation log sheet for solid samples (see attached) with the placement and contents of the vials on the tray.
3. Add 25 mg of sample (except for the method blank vials), 100 µl of 0.17 N MgSO₄, and 0.5 ml DIW to each vial. Record the date the MgSO₄ was made.
4. Place tray in an 80 °C oven and evaporate to dryness (usually overnight). Record the date and time the samples were placed in the oven and removed from the oven on the log sheet.
5. Transfer the vials from the plastic trays to the metal trays, keeping the vials in the exact same order as outlined on the sample preparation log sheet. Ash the samples at 550 °C in a muffle furnace for 3 hours and allow to cool overnight. A melt pellet (melting point of 550 °C) should be placed in an empty vial in an empty space on one of the trays to confirm that the furnace reached 550 °C. Record the date and time the samples were placed in the furnace and removed from the furnace and whether or not the pellet melted. Once cool, return the vials from the metal trays to the plastic trays, keeping the vials in the exact same order as outlined on the sample preparation log sheet.

6. Hydrolyze each sample with the addition of 5 ml of 0.24 N hydrochloric acid. Record the date and time the acid was added and the date the acid was made on the sample preparation log sheet.
7. Cap each sample tightly with polylined caps, shake using a vortexer, and put into an 80 °C oven overnight. After removing from the oven, allow to cool and shake again. Record the dates and times the vials were taken out of the oven and shaken on the sample preparation log sheet.
8. Analyze at a 1:20 dilution (100 µl of sample with 1900 µl DIW).

5- ANALYSIS

5.1- Autoanalyzer Reagents

All reagents are made with the high reagent-grade chemicals dissolved in DIW.

Wash water: The salinity of the wash water and the samples affects the shape of the peaks from the autoanalyzer, therefore we match the salinity of the wash water to the salinity of the samples. Samples with salinities of 15 ppt or less are run with nutrient-free DIW acidified with sulfuric acid (2.0ml/15L). Samples with salinities greater than 15 ppt are run with nutrient-free seawater acidified with sulfuric acid (3.0ml/15L). The nutrient-free seawater is obtained from the Gulf Stream and stored in carboys fitted with ammonia traps. Wash water are pumped directly from these carboys to the autoanalyzer.

Reagent Blank: This blank consists of the same analyte-free water (DIW or Gulf Stream seawater depending upon the salinity of the samples) used to prepare the working standards (see section 5.2).

Method Blank (digestion blank): A vial containing only the reagents used throughout the sample preparation protocol that is processed through the whole analytical procedure and analyzed with a specified sample set.

Total phosphorus analysis requires five reagents which are mixed just prior to the analysis to make a working reagent:

Antimony potassium tartrate: 0.75 g antimony potassium tartrate is dissolved in 250 ml DIW. This solution must be stored at 4 °C in a dark bottle.

Ammonium molybdate: 20 g ammonium molybdate is dissolved in 500 ml DIW. This solution must be stored at 4 °C in a plastic bottle.

Sulfuric acid solution: 140 ml conc. sulfuric acid is added to 900 ml DIW.
(Use caution when making acid solutions. Always add acid to water. The mixing of acid in water may generate heat.)

Ascorbic acid: 6.0 g ascorbic acid is dissolved in 200 ml acetone and 200 ml DIW. This solution must be stored at 4 °C for 1 week maximum.

Sodium dodecyl sulfate (SDS), 15% w/w: 15 g sodium dodecyl sulfate is added to 85 ml DIW.

Working mixed reagent: Combine 50 ml sulfuric acid solution, 5 ml antimony potassium tartrate solution, 15 ml ammonium molybdate, 30 ml ascorbic acid, and 2 ml SDS, in that order. This combined reagent must be prepared before each run.

5.2- Total Phosphorus Standards

The primary standard for total phosphorus is the same as that used for soluble reactive phosphate.

Phosphate primary standard, 1000 μM : 0.1360 g of previously dried (1 hour at 105 $^{\circ}\text{C}$) potassium dihydrogen phosphate is dissolved in 1 L DIW. Add 2 ml chloroform. Final concentration is the equivalent of 1.0 $\mu\text{moles/ml}$.

Phosphate QC check standard, 0.50 μM : An S3 working standard is prepared using a Phosphate primary standard (1000 μM) from a second source.

Citrus Leaf standard : This NIST certified standard reference material (SRM) is used as a QC check sample for solids. It is prepared as a normal solid sample passing through the whole analytical procedure. The true concentration is 1300 $\mu\text{g/g}$.

Working standards are prepared from the primary standard in DIW or Gulf Stream seawater depending upon the salinity of the samples. For water samples with salinities of 15 ppt or less, standards are prepared in DIW. Gulf Stream seawater is used for water samples with salinities greater than 15 ppt. The working standards are made to bracket the expected concentration of the samples as shown in Table 1. A log book is kept by the total phosphorus autoanalyzer. In the log book record (see attached) the slope and correlation coefficient of the calibration curve, the water matrix (freshwater or seawater), the instrument range setting, the technician's initials, and the preparation dates of the primary phosphorus standard and five reagents.

5.3- Autoanalyzer Instrument Parameters

Flowcell: 5.5 mm

Filter: 660 nm

Heater temperature: 45 $^{\circ}\text{C}$

Sample time: 20 sec

Wash time: 40 sec

Peristaltic pump setting: 60

Tubing:

black / black (air): 0.32 cc/m

yellow / blue (sample + water): 1.4 cc/m

blue / blue (wash water): 1.6 cc/m

orange / white (mixed reagent): 0.23 cc/m
red / red (waste): 0.80 cc/m

5.4- Autoanalyzer Calibration and Operation

After power is turned on to all units and the tubes are reconnected to the rollers, start wash water (acidified DIW or seawater, depending on the samples to be analyzed) and mixed reagent flowing through the autoanalyzer. Then flush the instrument for at least 10 minutes or until the baseline is stable, then press autozero. The computer table is then created with the samples to be loaded.

Each run of the autoanalyzer begins with the running of an S5 cup (SYNC) then two low standard S1 cups to calculate the carryover (CO) followed by a reagent blank (RB). A complete set of the 6 working standards (S1, S2,..., and S6) described in Table 1 are then run. Following the standard curve there are two blanks (RB), the QC Check standard that is an S2 prepared from a second source (Internal Calibration Verification, ICV) and the initial S4 (Continuing Calibration Verification, CCV) .

A reagent blank sample (RB) and an S4 (CCV) are run every 10 injections in the autoanalyzer run to monitor baseline and intra-run calibration drifts. Method (digestion) blank and matrix spike samples are run by duplicate at any time during the run. Right before injecting the matrix spike sample, the same corresponding unspiked sample must be run. For solid samples the citrus leaves QC check sample (SRM) is injected by duplicate at any time during the run.

5.5- Autoanalyzer Shutdown

The following reagents are needed:

Sodium hydroxide (NaOH), 1 N: 40 g of sodium hydroxide is added to 1 L of DIW.

Hydrochloric acid (HCl), 10% v/v: 100 ml of conc. hydrochloric acid in 1 L total volume.

After the last samples are analyzed, the instrument should be flushed with fresh wash water for 5 min, 1 N NaOH for 5 min, 10% HCl for 5 min, DIW for 5 min, and air for 5 min. Turn off the power at the power strip (heat bath remains on all the time). Remove the tubes from the rollers.

5.6- Autoanalyzer Preventative Maintenance

All spills are immediately cleaned up. Following solid sample analyses, NaOH and HCl are flushed through the tubing longer (10 min each) or are pushed through with a syringe for greater pressure. Any worn tubing is replaced immediately.

6- CALCULATIONS

Alpkem Win Flow V3 Software is used. Absorbance is directly proportional to phosphorus concentration and is measured as peak height units. The peak heights on the output have been corrected by the software for carry over and for intrarun and baseline drifts. The slope, intercept, and correlation coefficient are calculated by the software by first order regression (least square method). The value for any given sample is then calculated by (peak height units - intercept)/slope. The intercept will impart a false concentration to all the values (i.e. on the low scale, the calculation will result in blanks with 0 peak height units having around a 0.07 μM concentration). As the software is not able to automatically correct for this, the false blank value must then be subtracted from all results. The correction will be done in the data entry of the values.

For solids, the sample weight is entered in the computer so the software could make the appropriate corrections in the calculation procedure.

7- QA/QC AND CORRECTIVE ACTION

The standard curve should have a correlation coefficient ≥ 0.995 . A failing correlation coefficient invalidates the run and suggests the need for either preventative maintenance on the autoanalyzer or re-making of the standards.

The carry over should be $\leq 5\%$. The initial CCV should be $\pm 10\%$ (5% is the warning level) of the expected value and the subsequent CCVs throughout the run should be $\pm 10\%$ (5% is the warning level) of the initial CCV. Equipment, reagent and method blank total phosphorus concentrations should be less than that corresponding to the S1 standard. QC check standards, Citrus leaves SRM, and Matrix spikes should be within the SERP historically generated control limits. The precision is monitored with matrix spike duplicates (Relative Percent Difference [RPD] should be less than 20%) and with, at least, one sample duplicate every ten. Any samples and runs which do not meet these criteria need to be reanalyzed. If a problem continues, preventative maintenance on the autoanalyzer may be necessary. Samples that are out-of-range of the standard curve are diluted and reanalyzed.

Table 1. Working standards for total phosphorus determination.

| Working Standard | Volume of Primary Standard (μl) in 100 ml of DIW or Seawater | Phosphorus Standard Concentration (μM) |
|----------------------------|---|---|
| LOW STANDARD CURVE | | |
| S1 | 10 | 0.10 |
| S2 | 50 | 0.50 |
| S3 | 100 | 1.00 |
| S4 | 150 | 1.50 |
| S5 | 200 | 2.00 |
| High Standard Curve | | |
| S1 | 125 | 1.25 |

| Working Standard | Volume of Primary Standard (µl) in 100 ml of DIW or Seawater | Phosphorus Standard Concentration (µM) |
|---|--|--|
| S2 | 250 | 2.50 |
| S3 | 500 | 5.00 |
| S4 | 750 | 7.50 |
| S5 | 1000 | 10.00 |
| Standard Curve for Solid Samples | | |
| S1 | 125 | 3.87 µg/g |
| S2 | 250 | 7.74 µg/g |
| S3 | 500 | 15.48 µg/g |
| S4 | 750 | 23.22 µg/g |
| S5 | 1000 | 30.97 µg/g |

Total Phosphorus Sample Preparation Log Sheet for Water Samples

Total Phosphorus Sample Preparation Log Sheet for Solid Samples

Total Phosphorus Log Book Record

Changes/Reasons for Change:

Document Approved By:

 Ronald Jones, Ph. D
 SERP Director and Professor

 Date

 Doraida Diaz
 SERP Quality Assurance Officer

 Date